

VACCINES COMPRISING NUCLEOTIDE SEQUENCES ENCODING BOVINE HERPESVIRUS TYPE 1 G1, G111 AND GIV

CROSS REFERENCE TO RELATED APPLICATION

This application is a Divisional of application Ser. No. 07/921,1992, now U.S. Pat. No. 5,585,265, which is a continuation-in-part of U.S. patent application Ser. No. 07/805,524, filed Dec. 11, 1991, now abandoned which is a continuation-in-part of U.S. patent application Ser. No. 07/219,939, filed Jul. 15, 1988, now U.S. Pat. No. 5,151,267 both of which are incorporated herein by reference in its entirety and from which priority is claimed pursuant to 35 USC §120.

TECHNICAL FIELD

The present invention relates generally to the prevention of disease in cattle. More particularly, the instant invention is directed to the recombinant production of bovine herpesvirus type 1 antigens for use in subunit vaccines to protect cattle against bovine herpesvirus type 1 infection.

BACKGROUND

Bovine herpesvirus type 1 (BHV-1) is an economically significant pathogen of cattle. BHV-1, which is also known as infectious bovine rhinotracheitis virus, causes severe respiratory infections, conjunctivitis, vulvovaginitis, abortions, encephalitis, and generalized systemic infections. If an animal recovers from a primary infection, the virus remains in the host in a latent state. Reactivation of the virus can be provoked by certain endogenous or exogenous physical modifications in the animal, or experimentally by treatment of the animal with glucocorticoids like dexamethasone.

In an effort to control BHV-1 infections, killed virus and attenuated live-virus vaccines have been developed. While these vaccines appear to induce some level of protection in cattle, the level of immunity is well below that necessary to afford complete or near-complete protection. For example, the vaccines do not always prevent the establishment of a latent infection by a virulent field strain of BHV-1. Furthermore, the safety of the live-virus vaccines has been questioned. It has been shown recently that two live BHV-1 vaccine strains can be reactivated by the use of dexamethasone, indicating that at Least some BHV-1 vaccines can themselves establish a latent infection. See, e.g., Gerber et al. (1978) *Am. J. Vet. Res.* 39:753-760; Jericho et al. (1983) *Can. J. Com. Med.* 47:133-139; Pastoret et al. (1980) *Infect. Immun.* 29:483-488. Subunit vaccines, i.e. vaccines including select proteins separated from the whole virus, afford a method for overcoming the problems inherent in the use of live and attenuated virus vaccines.

Several polypeptides of BHV-1 have now been studied. Misra et al. (1981) *J. Virol.* 40:367-378, reports on the partial characterization of a number of BHV-1 polypeptides and their immunoprecipitation with antiserum. van Drunen Littel-van den Hurk et al. (1984) *Virology* 135:466-479 and van Drunen Littel-van den Hurk et al. (1985) *Virology* 144:216-227 are directed to monoclonal antibodies developed against BHV-1 glycoproteins, and the ability of the monoclonal antibodies to neutralize virus and participate in antibody-dependent complement-mediated lysis in vitro. See also Collins et al. (1984) *J. Virol.* 52:403-409; Okazaki et al. (1986) *Virology* 150:260-264. van Drunen Littel-van

den Hurk et al. (1985) *Virology* 144:204-215 is directed to the purification of BHV-1 glycoproteins by immunoabsorbent chromatography and the production of antiserum in rabbits. van Drunen Littel-van den Hurk et al. (1986) *J. Clin. Microbiol.* 23:274-282 is directed to in vitro immunoreactivity of purified BHV-1 glycoproteins and bovine antiserum. Okazaki et al. (1987) *Arch. Virol.* 92:17-26 pertains to in vitro studies of the reactivities of monoclonal antibodies against BHV-1 glycoproteins with infected cells. Babiuk et al. (1987) *Virology* 159:57-66 relates to the purification of gI, gIII and gIV from virus infected cell lysates. This reference also discloses that gI of BHV-1 corresponds to gB of herpes simplex virus (HSV); gIII corresponds to gC; and gIV corresponds to gD. Purified gI, gIII and gIV have been shown to induce high levels of neutralizing antibody in cattle and participate in antibody dependent cell cytotoxicity of BHV-1 cells. The purified glycoproteins were also shown to protect cattle from disease. Babiuk et al. (1987) *Virology* 159:57-66. van Drunen Littel-van den Hurk et al. (1990) *Vaccine* 8:358-368 confirmed the protectivity of gI, gIII and gIV and studied the epitope specificity of the immune response to the glycoprotein vaccines. Hughes et al. (1988) *Arch. Virol.* 103:47-60 identified three neutralizing antigenic domains on gIV.

None of the above art, however, discloses the recombinant production of BHV-1 glycoproteins for use in recombinant vaccines. Mayfield et al. (1983) *J. Virol.* 47:259-264 discloses the cloning of a BHV-1 strain and a restriction map. Fitzpatrick et al. (1989) *Virology* 173:46-57, describe the nucleotide sequence of gIII. Pahl et al. (1987) *J. Virol.* 61:315-325 describe the recombinant expression of a glycoprotein from the human pathogen HSV-1. There was no demonstration, however, that the recombinant polypeptide from the human virus was, in fact, protective in a human host. See also PCT Pub. No. WO88/02634; U.S. Pat. Nos. 4,661,349; 4,642,333.

Fitzpatrick et al. (1988) *J. Virol.* 62:4239-4248 describe the expression of gI and gIII in murine LMTK- cells. The transfected cells were shown to stimulate the production of neutralizing antibodies in mice. Fitzpatrick et al. (1990) describe the expression of deleted, truncated and hybrid forms of gI and gIII in murine LMTK-cells and epitope mapping of the same. Tikoo et al. (1990) *J. Virol.* 64:5132-5142 disclose the mapping, cloning and sequencing of BHV-1 gIV, as well as the expression of gIV in bovine cells. van Drunen Littel-van den Hurk et al. (1989) *J. Virol.* 63:2159-2168 disclose the expression of gI and gIII in a vaccinia virus vector. The recombinant vectors elicited a neutralizing antibody response in cattle immunized with the same. van Drunen Littel-van den Hurk et al. (January 1991) *J. Virol.* 65:263-271 describe the expression of gIV by recombinant baculovirus. This disclosure was based in part on the present invention. Cattle immunized with recombinant gIV raised neutralizing antibodies thereto.

DISCLOSURE OF THE INVENTION

It has been discovered that recombinant subunit vaccines, based on selected BHV-1 glycoproteins, will protect cattle from disease. These vaccines are particularly useful in protecting cattle from the shipping fever complex syndrome which often includes infection by BHV-1. Surprising, these subunit vaccines are substantially more protective than prior art killed virus and attenuated live-virus vaccines. The recombinant subunit vaccines do not suppress the immunological response to other components often found in multivalent shipping fever vaccines. Further, the recombinant subunit vaccines of the present invention also eliminate the